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X-ray powder diffraction: A powerful analysis tool for industrial protein production

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X-ray powder diffraction (XRPD) offers a method of characterizing a crystalline protein suspension using home laboratory X-ray sources [1–2]. For well diffracting samples XRPD data can be collected within 30 minutes, which is appealing for industrial applications. In industry, enzymes and proteins are produced and handled in high concentrations, which can in turn cause problems for the processes due to protein precipitation in the production pipeline. Using a known library of structures, XRPD is particularly useful for identification of the different crystal forms present in a suspension, by fitting calculated patterns of known single crystal forms to the observed XRPD pattern. For this purpose we have developed a streamlined program for calculation of powder diffraction patterns from coordinate files taking into account disordered solvent, peak asymmetry and background handling [3].

XRPD was applied to a suspension from a large-scale industrial production of the widely used *Bacillus lentus* subtilisin containing both small crystals (~30 µm) as well as microcrystals (< 1 µm). A dominant crystal form was identified by XRPD, but two other different crystal forms were found by a complementary single crystal micro-diffraction analysis of the larger single crystals present in the sample [4]. The study demonstrated the presence of at least three different microcrystalline forms, and serves as a reminder that when a crystal is picked out from a batch crystallization for single crystal analysis, it might not be representative of the bulk microcrystalline material in the sample.

To estimate the fraction of the different crystal forms in production samples with significant polymorphism, a further XRPD study was performed on binary mixtures of different lysozyme and subtilisin crystal forms. Quantitative XRPD generally requires careful sample preparation, with uniformly sized and randomly ordered fine powders to accurately obtain the relative composition of crystal forms. Working with protein slurries leads to further challenges in terms of varying crystal density in the suspensions as well as type of suspension medium. After careful optimisation of suspension medium, and crystallite size, the relative composition of crystal forms can be determined within 10 %.

In conclusion this work demonstrates the value of in-house XRPD as an analysis tool in industrial enzyme production, and its potential to greatly help troubleshooting the production process and to provide valuable information for further refining the manufacturing of enzymes and proteins.

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